



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

August 15, 2014

MEMORANDUM

SUBJECT: Efficacy Review for CDQ
EPA Reg. No. 5813-79
DP Barcode: 420226

FROM: Marcus Rindal, Microbiologist
Product Science Branch
Antimicrobials Division (7510P) *MRD 8.15.14*

THRU: Mark Perry, Team Leader
Product Science Branch
Antimicrobials Division (7510P) *MSP*

TO: Velma Noble, PM 32/Drusilla Copeland
Regulatory Management Branch II
Antimicrobials Division (7510P)

APPLICANT: The Clorox Company
c/o PS&RC
P.O. Box 493
Pleasanton, CA 94566-0803

FORMULATION FROM LABEL:

<u>Active Ingredient(s)</u>	<u>% by wt.</u>
n-Alkyl (C14, 60%; C16, 30%; C12, 5%; C18, 5%) Dimethyl Benzyl Ammonium Chloride.....	0.184%
n-Alkyl (C12, 68%; C14, 32%) Dimethyl Ethylbenzyl Ammonium Chloride.....	0.184%
Other ingredients.....	99.632%
Total.....	100.00%

I. BACKGROUND

With this action, the registrant is seeking an amendment to the ready-to-use towelette (wipe) product (CDQ; EPA Reg. No. 5813-79) that adds new organism claims and revises directions for use and marketing claims. It is a non-food contact, sanitizing and disinfecting wipe for hard non-porous surfaces. Studies were conducted by ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

This data package contained: a letter from the registrant dated April 15, 2014, Certification with Respect to Citation of Data (EPA Form 8570-34), Data Matrix (EPA Form 8570-35), a proposed product label (dated 4/15/14; 20 pages), and 23 efficacy studies (MRID 493634-01 through -23) with a Statement of No Data Confidentiality Claims embedded in each MRID.

The towelette product, CDQ (EPA Reg. No. 5813-79), is an EPA-approved, ready-to-use fungicide and bacteriostat for use on hard, non-porous surfaces in household, commercial, institutional, industrial, food preparation, animal care, and hospital or medical environments. The product is the secondary to Spruce-Ups, EPA Reg. No. 5813-58. The applicant requested to amend the registration of this product to add new claims for effectiveness as a hospital disinfectant, with sanitization activity for non-food contact surfaces. Additional viruses and bacteria were also being added to the label. Studies were conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

II. USE DIRECTIONS

The product, CDQ is intended to be used as a disinfecting or sanitizing read-to-use wipe on hard, non-porous, non-food contact surfaces in; homes, offices, industrial buildings, hotels, motels, hospitals, schools, day cares, nursing homes, cars, airplanes, playgrounds etc. Surfaces to be cleaned include metal, plastic, hardwood, vinyl, aluminum, stainless steel, granite, acrylic, glass, fiberglass, and rubber. The product is not for use on dishes, glassware, or eating utensils and is not recommended for use on unpainted wood.

The following use directions were given on the proposed label:

TO SANITIZE: [Use to sanitize] [/deodorize] hard, nonporous surfaces. Use the soft –or– smooth side of wipe to sanitize/deodorize. Wipe surface; use enough wipes for treated surfaces to remain visibly wet for 10 seconds. Let surface dry. For highly soiled surfaces, clean excess dirt first.

TO DISINFECT [AND DEODORIZE]: [Use to disinfect] hard, nonporous surface: Wipe surface [to be disinfected]; use enough wipes for treated surface to remain visibly wet for 4 minutes. Let surface dry. For highly soiled surfaces, clean excess dirt first. For surfaces that may come in contact with food, a potable water rinse is required. This product is not for use on dishes, glassware, or eating utensils.

III. AGENCY STANDARDS

Antimicrobial Products for Use on Hard Surfaces Using Pre-saturated or Impregnated Towelettes: Towelette products represent a unique combination of antimicrobial chemical and applicator, pre-packaged as a unit in fixed proportions. As such, the complete product, as offered for sale, should be tested according to the directions for use to ensure the product's effectiveness in treating hard surfaces. The standard test methods available for hard surface disinfectants and sanitizers, if followed exactly, would not closely simulate the way a towelette product is used. Agency guidelines recommend that a simulated-use test be conducted by modifying the standard test methods. Agency guidelines further recommend that instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the slides after a specified holding time. Performance standards of the standard test methods must be met. These Agency standards are presented in EPA Pesticide Assessment Guidelines, Subdivision G, §91-2(h), Pre-saturated or impregnated towelettes; and the April 12, 2001 EPA Memorandum, Draft Interim Guidance for Non-Residual Sanitization of Hard Inanimate Food Contact Surfaces Using Pre-Saturated Towelettes.

Sanitizer Test (for inanimate, non-food contact surfaces): The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 493634-01, "Pre-Saturated Towelettes for Hard Surface Disinfection," Test Organism: *Pseudomonas aeruginosa*, (ATCC 15442) for product CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz36. Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – March 11, 2014. Project Number A16285.

This study was conducted against *Pseudomonas aeruginosa*, (ATCC 15442). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0014

and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz36. These were tested using ATS Laboratory Protocol No. CX18010914.TOW.2 (copy provided). The product was received as ready to use saturated towelettes. A 10 µL aliquot of a thawed cryovial of stock culture was transferred to an initial 10 mL tube of Nutrient broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 mL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). This was followed by two additional daily transfers. The final test culture was incubated for 48-54 hours at 35-37°C. On the day of use, the pellicle was carefully aspirated from the culture by vacuum aspiration and discarded. The culture was visually inspected to ensure no pellicle fragments were present then was diluted by combining 1.0 mL of test organism with 1.0 mL of growth medium and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 54.2-55.4% relative humidity. Carriers were used within 2 hours of drying. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 30 seconds at room temperature (21.0°C) and 9.1% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported.

2. **MRID 493634-02, "Pre-Saturated Towelettes for Hard Surface Disinfection,"**
Test Organism: *Pseudomonas aeruginosa*, (ATCC 15442) for product CDQ,
EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9,
lotion of F2012.0014, Batch 13wz38. Study conducted at ATS Labs by Gracia
Schroeder, B.S. Study completion date – March 12, 2014. Project Number
A16300.

This study was conducted against *Pseudomonas aeruginosa*, (ATCC 15442). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz38. These were tested using ATS Laboratory Protocol No. CX18010914.TOW.5 (copy provided). The product was received as ready to use saturated towelettes. A 10 µL aliquot of a thawed cryovial of stock culture was transferred to an initial 10 mL tube of Nutrient broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 mL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). This was followed by two additional daily transfers. The final test culture was incubated for 48-54 hours at 35-37°C. On the day of use, the pellicle was carefully

aspirated from the culture by vacuum aspiration and discarded. The culture was visually inspected to ensure no pellicle fragments were present then was diluted by combining 1.0 mL of test organism with 1.0 mL of growth medium and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 μ L of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 50.4-55.2% relative humidity. Carriers were used within 2 hours of drying. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 30 seconds at room temperature (21.0°C) and 8.8% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth to neutralize. All subcultures were incubated for 48 \pm 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

3. MRID 493634-03, "Pre-Saturated Towelettes for Hard Surface Disinfection,"
Test Organism: *Salmonella enterica* (ATCC 10708), for product CDQ, EPA
Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of
F2012.0014, Batch 13wz38. Study conducted at ATS Labs by Jill Ruhme, B.S.
Study completion date – March 11, 2014. Project Number A16296.

This study was conducted against *Salmonella enterica* (ATCC 10708). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz38. These were tested using ATS Laboratory Protocol No. CX18010914.TOW.6 (copy provided). The product was received as ready to use saturated towelettes. A 10 μ L aliquot of a thawed cryovial of stock culture was transferred to an initial 10 mL tube of Synthetic broth growth medium, mixed and incubated for 24 \pm 2 hours at 35-37°C. A 10 mL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). This was followed by two additional daily transfers. The final test culture was incubated for 48-54 hours at 35-37°C. On the day of use, the culture was diluted by combining 2.0 mL of test organism with 2.0 mL of growth medium and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 μ L of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 53.0-54.0% relative humidity. Carriers were used within 2 hours of drying. Each towelette

was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 30 seconds at room temperature (21.5°C) and 10.3% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

4. MRID 493634-04, "Pre-Saturated Towelettes for Hard Surface Disinfection,"
Test Organism: *Salmonella enterica* (ATCC 10708), for product CDQ, EPA
Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of
F2012.0014, Batch 13wz36. Study conducted at ATS Labs by Joshua
Luedtke, M.S. Study completion date – March 11, 2014. Project Number
A16297.

This study was conducted against *Salmonella enterica* (ATCC 10708). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz36. These were tested using ATS Laboratory Protocol No. CX18010914.TOW.3 (copy provided). The product was received as ready to use saturated towelettes. A 10µL aliquot of a thawed cryovial of stock culture was transferred to an initial 10 mL tube of Synthetic broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 mL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). This was followed by two additional daily transfers. The final test culture was incubated for 48-54 hours at 35-37°C. On the day of use, the culture was diluted by combining 2.0 mL of test organism with 2.0 mL of growth medium and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 53.0-54.0% relative humidity. Carriers were used within 2 hours of drying. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 30 seconds at room temperature (22.2°C) and 9.3% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence

of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

- 5. MRID 493634-05, "Pre-Saturated Towelettes for Hard Surface Disinfection,"**
Test Organism: *Staphylococcus aureus* (ATCC 6538), for product CDQ, EPA
Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of
F2012.0014, Batch 13wz36. Study conducted at ATS Labs by Mary J. Miller,
M.T. Study completion date – March 12, 2014. Project Number A16134.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz36. These were tested using ATS Laboratory Protocol No. CX18010814.TOW.1 (copy provided). The product was received as ready to use saturated towelettes. A 10 μ L aliquot of a thawed cryovial of stock culture was transferred to an initial 10 mL tube of Synthetic broth growth medium, mixed and incubated for 24 \pm 2 hours at 35-37°C. A 10 mL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). This was followed by four additional daily transfers. The final test culture was incubated for 48-54 hours at 35-37°C. On the day of use, the culture was diluted by combining 1.0 mL of test organism with 11.0 mL of growth medium (protocol deviation = dilution of 1:12) and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 μ L of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 55.8-55.9% relative humidity. Carriers were used within 2 hours of drying. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 35 seconds at room temperature (20.8°C) and 8.2% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Lethen Broth to neutralize. All subcultures were incubated for 48 \pm 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments and deviations reported in this study were reviewed and found to be acceptable.

- 6. MRID 493634-06, "Pre-Saturated Towelettes for Hard Surface Disinfection,"**
Test Organism: *Staphylococcus aureus* (ATCC 6538), for product CDQ, EPA
Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of
F2012.0014, Batch 13wz38. Study conducted at ATS Labs by Mary J. Miller,
M.T. Study completion date – March 12, 2014. Project Number A16144.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz38. These were tested using ATS Laboratory Protocol No. CX18010814.TOW.2 (copy provided). The product was received as ready to use saturated towelettes. A 10 μ L aliquot of a thawed cryovial of stock culture was transferred to an initial 10 mL tube of Synthetic broth growth medium, mixed and incubated for 24 \pm 2 hours at 35-37°C. A 10 mL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). This was followed by four additional daily transfers. The final test culture was incubated for 48-54 hours at 35-37°C. On the day of use, the culture was diluted by combining 1.0 mL of test organism with 11.0 mL of growth medium (protocol deviation = dilution of 1:12) and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 μ L of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 55.1-55.2% relative humidity. Carriers were used within 2 hours of drying. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 35 seconds at room temperature (21.3°C) and 8.0% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Lethen Broth to neutralize. All subcultures were incubated for 48 \pm 2 hours at 35-37°C. Subcultures were stored at 2-8°C for two days prior to examination. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments and deviations reported in this study were reviewed and found to be acceptable.

- 7. MRID 493634-07, "Pre-Saturated Towelettes for Hard Surface Disinfection,"**
Test Organism: *Bordetella pertussis* (ATCC 12743), for product CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38. Study conducted at ATS Labs by Joshua Luedtke, M.S. Study completion date – March 11, 2014. Project Number A16233.

This study was conducted against *Bordetella pertussis* (ATCC 12743). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz36 and Batch 13wz38. These were tested using ATS Laboratory Protocol No. CX18010914.TOW.14 (copy provided). The product was received as ready to use saturated towelettes. From stock, a sufficient number of Bordet Gengou Agar plates were inoculated with the test organism and incubated for three days at 35-37°C. Following incubation, the organism was suspended in Butterfield's buffer to match a 3.0 McFarland turbidity standard and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared

culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 64-65% relative humidity. Carriers were used within 2 hours of drying. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 30 seconds at room temperature (22.2°C) and 27.6% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Lethen Broth + 0.07% Lechithin + 0.5% Tween 80 to neutralize. After vortexing, the entire volume of the subculture broths were individually transferred to a filter membrane (0.45 µm porosity) pre-wetted with 10 mL of sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL sterile saline and then was removed from the filter unit and placed on the surface of a Bordet Gengou Agar plate for organism recovery. All subcultures were incubated for 4 days at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Note: No protocol amendments or deviations were reported in this study.

8. **MRID 493634-08, "Pre-Saturated Towelettes for Hard Surface Disinfection,"**
Test Organism: *Enterobacter aerogenes* (ATCC 13048), for product CDQ,
EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9,
lotion of F2012.0014, Batches 13wz36 and 13wz38. Study conducted at ATS
Labs by Joshua Luedtke, M.S. Study completion date – March 11, 2014.
Project Number A16232.

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batch 13wz36 and Batch 13wz38. These were tested using ATS Laboratory Protocol No. CX18010914.TOW.13 (copy provided). The product was received as ready to use saturated towelettes. A loop of stock culture was transferred to an initial 10 mL tube of Tryptic Soy Broth growth medium, mixed and incubated for 24±2 hours at 25-30°C. A 10 mL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (1st daily transfer). This was followed by three additional daily transfers. The final test culture was incubated for 48-54 hours at 25-30°C. On the day of use, the culture was diluted by combining 1.0 mL of test organism with 2.0 mL of sterile growth medium and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 52.7-53.8% relative humidity.

Carriers were used within 2 hours of drying. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 30 seconds at room temperature (21.9°C) and 17.1% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No protocol amendments or deviations were reported in this study.

**9. MRID 493634-09 "Pre-Saturated Towelettes for Hard Surface Disinfection,"
Test Organism: Methicillin Resistant *Staphylococcus aureus*-MRSA (ATCC 33592), for product CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013, Batches 12DMK1 and 12DMK2. Study conducted at ATS Labs by Nicole Albert, B.S. Study completion date – March 14, 2012. Project Number A12958.**

This study was conducted against Methicillin Resistant *Staphylococcus aureus*-MRSA (ATCC 33592). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013, Batch 12DMK1 and Batch 12DMK2. These were tested using ATS Laboratory Protocol No. CX18030612.TOW.19 (copy provided). The product was received as ready to use saturated towelettes. From a stock slant, an initial 10 mL tube of Nutrient Broth growth medium was inoculated, mixed, and incubated, followed by performing a minimum of three but less than thirty daily subculture transfers. The final subculture was incubated for 40-54 hours at 35-37°C. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (three inch by one inch), each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 33 minutes at 35-37°C and at 40-43% relative humidity. Carriers were used within 2 hours of drying. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 3 minutes 45 seconds at room temperature (25.7°C) and 17.0% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to 40 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for two days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Antimicrobial susceptibility testing was performed by ATS Lab to verify the antibiotic resistance of the MRSA test organism. The Kirby Bauer susceptibility assay was performed utilizing a representative test organism from the day of testing. After incubation at 35-37°C for ≥ 24 hours, the zone (diameter) of inhibition showing no visible growth indicating resistance of the test organism.

Note: Protocol amendments and deviations reported in this study were reviewed and found to be acceptable.

10. MRID 493634-10 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection," Virus: Avian Influenza A (H3N2) Reassortant virus, for product CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 by Shanen Conway, B.S. Study conducted at ATS Labs. Study completion date – March 13, 2014. Project Number A16155.

This study was conducted against Avian Influenza A (H3N2) Reassortant virus, ATCC VR-2072, Strain A/Washington/897/80 x A/Mallard/New York/6750/78. Two batches of test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 were tested using ATS Laboratory Protocol No. CX18011714.AFLU.1 (copy provided). The product was received as ready to use saturated towelettes. The host cell line was canine kidney (MDCK) cells obtained from ATCC, Manassas, VA (ATCC CCL-34). Cultures were maintained and used at 36-38°C in a humidified atmosphere of 5-7% CO₂. Test medium used to maintain the cell cultures was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% BSA fraction V, 100 units/mL penicillin, 10 µg /mL gentamicin, and 2.5 µg/mL amphotericin B. On the day of testing, three aliquots of stock virus were thawed and maintained at refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of three 150 x 15 mm sterile glass petri dishes and dried at 20.0°C for 20 minutes at 50% relative humidity. The test substance Spruce-Ups consisting of single use towelettes impregnated with the active ingredient, one towelette was used to wipe one glass petri dish. For each batch of test substance, using sterile gloves, the dried virus film on the surface of two inoculated glass petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The petri dishes were covered and held at 20.0°C for 3 minutes and 30 seconds. Following the exposure time, a 2.00 mL aliquot of test medium was added to each petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10⁻¹ dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10⁻¹ dilution) was immediately titered by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The MDCK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

11. MRID 493634-11 "Virucidal Efficacy of Pre-Saturated Towelettes for Use on Inanimate Environmental Surfaces," Virus: Avian Influenza A (H5N1) virus, Strain VNH5N1-PR8/CDC-RG, CDC # 2006719965, for product CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013, Batches 12DMK1 and 12DMK2 by Dawn Pierson, B.S. Study conducted at ATS Labs. Study completion date – May 22, 2012. Project Number A12953.

This study was conducted against VNH5N1-PR8/CDC-RG, CDC # 2006719965 strain of Avian Influenza A (H5N1) virus obtained from the CDC, Atlanta, GA. Two batches of test substance CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013, Batches 12DMK1 and 12DMK2 were tested using ATS Laboratory Protocol No. CX18030612.AFLU (copy provided). The product was received as ready to use saturated towelettes. The host cell line was Rhesus monkey kidney (RMK) cells obtained from ViroMed Laboratories, Inc. Cultures were maintained and used at 36-38°C in a humidified atmosphere of 5-7% CO₂. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 1% (v/v) heat inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 ng/mL amphotericin B. On the day of testing, three aliquots of stock virus were thawed and maintained at refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of three 150 x 15 mm sterile glass petri dishes and dried at 22.0°C for 20 minutes at 28.7% relative humidity. The test substance Spruce-Ups consisting of single use towelettes impregnated with the active ingredient, one towelette was used to wipe one glass petri dish. For each batch of test substance, using sterile gloves, the dried virus film on the surface of two inoculated glass petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The petri dishes were covered and held at 22.0°C for 3 minutes and 45 seconds. Following the exposure time, a 2.00 mL aliquot of test medium was added to each petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10⁻¹ dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10⁻¹ dilution) was immediately titrated by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The RMK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

12. MRID 493634-12 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection," Virus: Avian Influenza A (H7N9) virus, Strain wildtype

A/Anhui/1/2013, CDC #2013759189, for product CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 by Shanen Conway, B.S. Study conducted at ATS Labs. Study completion date – March 13, 2014. Project Number A16157.

This study was conducted against Avian Influenza A (H7N9) wildtype A/Anhui/1/2013, CDC #2013759189. Two batches of test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 were tested using ATS Laboratory Protocol No. CX18011714.AFLU.3 (copy provided). The product was received as ready to use saturated towelettes. The host cell line was canine kidney (MDCK) cells obtained from ATCC, Manassas, VA (ATCC CCL-34). Cultures were maintained and used at 36-38°C in a humidified atmosphere of 5-7% CO₂. Test medium used to maintain the cell cultures was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 µg/mLTPCK-trypsin, 25 mM HEPES, 0.2% BSA fraction V, 100 units/mL penicillin, 10 µg /mL gentamicin, and 2.5 µg/mL amphotericin B. On the day of testing, three aliquots of stock virus were thawed and maintained at refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of three 150 x 15 mm sterile glass petri dishes and dried at 21.0°C for 20 minutes at 56% relative humidity. The test substance Spruce-Ups consisting of single use towelettes impregnated with the active ingredient, one towelette was used to wipe one glass petri dish. For each batch of test substance, using sterile gloves, the dried virus film on the surface of two inoculated glass petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The petri dishes were covered and held at 21.0°C for 3 minutes and 30 seconds. Following the exposure time, a 2.00 mL aliquot of test medium was added to each petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10⁻¹ dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10⁻¹ dilution) was immediately titered by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The MDCK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

13. MRID 493634-13 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection," Virus: Canine Distemper virus, for product CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 by Shanen Conway, B.S. Study conducted at ATS Labs. Study completion date – March 13, 2014. Project Number A16154.

This study was conducted against Lederle strain of Canine Distemper virus

obtained from the ATCC, Manassas, VA (ATCC VR-128). Two batches of test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 were tested using ATS Laboratory Protocol No. CX18011714.CDIS (copy provided). The product was received as ready to use saturated towelettes. The host cell line was Vero cells obtained from the ATCC, Manassas, VA (ATCC CCL-81). Cultures were maintained and used at 36-38°C in a humidified atmosphere of 5-7% CO₂. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 ng/mL amphotericin B. On the day of testing, three aliquots of stock virus were thawed and maintained at refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of three 150 x 15 mm sterile glass petri dishes and dried at 20.0°C for 20 minutes at 40% relative humidity. The test substance Spruce-Ups consisting of single use towelettes impregnated with the active ingredient, one towelette was used to wipe one glass petri dish. For each batch of test substance, using sterile gloves, the dried virus film on the surface of two inoculated glass petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The petri dishes were covered and held at 20.0°C for 3 minutes and 30 seconds. Following the exposure time, a 2.00 mL aliquot of test medium was added to each petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10⁻¹ dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10⁻¹ dilution) was immediately titrated by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The Vero cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

Note: Test substance cytotoxicity was observed in the 10⁻¹ dilution of both batches. The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤1.50 log₁₀ for both batches. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was ≥4.00 log₁₀ for Batch 13wz36 and ≥3.00 log₁₀ for Batch 13wz38.

14. MRID 493634-14 "Virucidal Efficacy of Pre-Saturated Towelettes for Use on Inanimate Environmental Surfaces" Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus for product CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013, Batches 12DMK1 and 12DMK2 by Mary J. Miller, M.T. Study conducted at ATS Labs. Study completion date – May 23, 2012. Project Number A12976.

This study was conducted against duck Hepatitis B virus used as a surrogate virus

for human Hepatitis B virus. The 10/29/11 strain of the duck Hepatitis B virus (DHBV) used in this assay was obtained from Hepadnavirus Testing Inc., Palo Alto, CA and consists of duck Hepatitis B virus serum obtained from congenially infected ducklings. Two batches of test substance CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013, Batches 12DMK1 and 12DMK2 were tested using ATS Laboratory Protocol No. CX18031912.DHBV (copy provided). The product was received as ready to use saturated towelettes. The host cell line was hepatocytes from ducklings verified to be free of the test virus and less than seven days old. Hepatocytes were obtained by an *in situ* perfusion of the duck liver utilizing S-MEM medium containing collagenase then seeded into sterile twelve well disposable tissue culture labware. Cultures were maintained and used at 36-38°C in a humidified atmosphere of 5-7% CO₂. Test medium used to maintain the cell cultures was Leibovitz L-15 medium supplemented with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin and 100 units/mL penicillin. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of three 150 x 15 mm sterile glass petri dishes and dried at 20.0°C for 20 minutes at 50% relative humidity. The test substance Spruce-Ups consisting of single use towelettes impregnated with the active ingredient, one towelette was used to wipe one glass petri dish. For each batch of test substance, using sterile gloves, the dried virus film on the surface of two inoculated glass petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The petri dishes were covered and held at 21.0°C for 3 minutes and 45 seconds. Following the exposure time, a 2.00 mL aliquot of test medium was added to each petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10⁻¹ dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10⁻¹ dilution) was immediately titered by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The primary duck hepatocytes in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 9 days for the absence or presence of CPE and for viability. On the final day of incubation, the cultures were observed microscopically for test substance cytotoxicity and the cells were then fixed with ethanol. An indirect immunofluorescence assay was then performed using a monoclonal antibody specific for the envelope protein of the DHBV. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

Note: Test substance cytotoxicity was observed in the 10⁻¹ dilution of both batches. The neutralization control (non-virucidal level of the test substance) indicates that both batches of the test substance were neutralized at ≤1.50 log₁₀. Utilizing an MPN statistical program, the log reduction in viral liter is ≥3.18 and the standard error of the log reduction is 0.00.

15. MRID 493634-15 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection" Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus for product CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014,

Batches 13wz36 and 13wz38 by Mary J. Miller, M.T. Study conducted at ATS Labs. Study completion date – March 12, 2014. Project Number A16143.

This study was conducted against duck Hepatitis B virus used as a surrogate virus for human Hepatitis B virus. The 10/29/11 strain of the duck Hepatitis B virus (DHBV) used in this assay was obtained from Hepadnavirus Testing Inc., Palo Alto, CA and consists of duck Hepatitis B virus serum obtained from congenially infected ducklings. Two batches of test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 were tested using ATS Laboratory Protocol No. CX18012014.DHBV (copy provided). The product was received as ready to use saturated towelettes. The host cell line was hepatocytes from ducklings verified to be free of the test virus and less than seven days old. Hepatocytes were obtained by an *in situ* perfusion of the duck liver utilizing S-MEM medium containing collagenase then seeded into sterile twelve well disposable tissue culture labware. Cultures were maintained and used at 36-38°C in a humidified atmosphere of 5-7% CO₂. Test medium used to maintain the cell cultures was Leibovitz L-15 medium supplemented with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin and 100 units/mL penicillin. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of three 150 x 15 mm sterile glass petri dishes and dried at 20.0°C for 20 minutes at 40% relative humidity. The test substance Spruce-Ups consisting of single use towelettes impregnated with the active ingredient, one towelette was used to wipe one glass petri dish. For each batch of test substance, using sterile gloves, the dried virus film on the surface of two inoculated glass petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The petri dishes were covered and held at 20.0°C for 3 minutes and 30 seconds. Following the exposure time, a 2.00 mL aliquot of test medium was added to each petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10⁻¹ dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10⁻¹ dilution) was immediately titered by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The primary duck hepatocytes in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 9 days for the absence or presence of CPE and for viability. On the final day of incubation, the cultures were observed microscopically for test substance cytotoxicity and the cells were then fixed with ethanol. An indirect immunofluorescence assay was then performed using a monoclonal antibody specific for the envelope protein of the DHBV. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

16. MRID 493634-16 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection," Virus: Newcastle disease virus, for product CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 by Mary J. Miller, M.T. Study conducted at ATS Labs. Study completion date – March 12, 2014. Project

Number A16158.

This study was conducted against The B1, Hitchner or Blacksburg strain of Newcastle disease virus from the ATCC (ATCC VR-108). Two batches of test substance CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014, Batches 13wz36 and 13wz38 were tested using ATS Laboratory Protocol No. CX18011714.NEW (copy provided). The host cell line was chicken embryo fibroblast (CEF) cells originally obtained from Charles River and propagated at ATS Laboratory. Cultures were maintained and used at 36-38°C in a humidified atmosphere of 5-7% CO₂. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 5% (v/v) tryptose phosphate broth and 2.0 mM L-glutamine. On the day of testing, three aliquots of stock virus were thawed and maintained at refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of three 150 x 15 mm sterile glass petri dishes and dried at 20.0°C for 20 minutes at 50% relative humidity. The test substance Spruce-Ups consisting of single use towelettes impregnated with the active ingredient, one towelette was used to wipe one glass petri dish. For each batch of test substance, using sterile gloves, the dried virus film on the surface of two inoculated glass petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The petri dishes were covered and held at 20.0°C for 3 minutes and 30 seconds. Following the exposure time, a 2.00 mL aliquot of test medium was added to each petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10⁻¹ dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10⁻¹ dilution) was immediately titrated by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The CEF cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

Note: Test substance cytotoxicity was observed in the 10⁻¹ dilution of both batches. The neutralization control (nonvirucidal level of the test substance) indicates that the test substance was neutralized at $\leq 1.50 \log_{10}$ for both batches. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $\geq 3.25 \log_{10}$ for both batches.

17. MRID 493634-17, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)," Test Organism: *Klebsiella pneumoniae* (ATCC 4352) for product CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40, 13wz41 and 13wz44. Study conducted at ATS Labs by

Joshua Luedtke, M.S. Study completion date – March 12, 2014. Project Number A16231.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40, 13wz41, and 13wz44. These were tested using ATS Laboratory Protocol No. CX18010914.NFS.2 (copy provided). The product was received as ready to use saturated towelettes. From a stock slant, an initial 10 mL tube of Synthetic Broth growth medium was inoculated, mixed, and incubated, followed by performing a minimum of three daily subculture transfers, each incubated for 24±2 hours. The final subculture was incubated for 48-54 hours and was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual glass slide carriers (1" x 1") per product batch, each in a Petri dish matted with two pieces of filter, were inoculated with 0.02 mL (20.0 µL) of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 35 minutes at 35-37°C and at 40% relative humidity. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 10 seconds at 21.6°C temperature and 16.2% relative humidity. Following the exposure period, the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Brain Heart Infusion Broth + 0.14% Lecithin + 1.0% Tween 80. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto BAP agar plates and were incubated at 35-37°C for 48±4 hours. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments reported in this study were reviewed and found to be acceptable. No protocol deviations were reported in this study.

18. MRID 493634-18, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)," Test Organism: *Staphylococcus aureus* (ATCC 6538), for product CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40, 13wz41 and 13wz44. Study conducted at ATS Labs by Joshua Luedtke, M.S. Study completion date – March 12, 2014. Project Number A16230.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40, 13wz41, and 13wz44. These were tested using ATS Laboratory Protocol No. CX18010914.NFS.1 (copy provided). The product was received as ready to use saturated towelettes. From a stock slant, an initial 10 mL tube of Synthetic Broth growth medium was inoculated, mixed, and incubated, followed by performing a minimum of three daily subculture transfers, each incubated for 24±2 hours. The final subculture was incubated for 48-54 hours and was

mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual glass slide carriers (1" x 1") per product batch, each in a Petri dish matted with two pieces of filter, were inoculated with 0.02 mL (20.0 µL) of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide in each Petri dish. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry with lids slightly ajar for 35 minutes at 35-37°C and at 41% relative humidity. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 10 seconds at room temperature (21.6°C) and 16.2% relative humidity. Following the exposure period, the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated on Tryptic Soy Agar plates with 5% Sheep Blood (BAP). All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No amendments were made. Deviations reported in this study were reviewed and found to be acceptable.

19. MRID 493634-19, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)," Test Organism: *Campylobacter jejuni* (ATCC 29428), for product CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, Lotion of F2012.0024, Batches 13wz40 and 13wz41. Study conducted at ATS Labs by Joshua Luedtke, M.S. Study completion date – March 12, 2014. Project Number A16292.

This study was conducted against *Campylobacter jejuni* (ATCC 29428). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, Lotion of F2012.0024, Batch 13wz40 and Batch 13wz41. These were tested using ATS Laboratory Protocol No. CX18010914.NFS.3 (copy provided). The product was received as ready to use saturated towelettes. A stock plate of the test organism was inoculated to Tryptic Soy Agar with 5% Sheep Blood agar plates (BAP) and incubated for 2-5 days at 35-37°C under microaerophilic conditions in a CampyPak™ Plus pack. From there, multiple BAP agar plates were inoculated and incubated for 2 days at 35-37°C in a CampyPak™ Plus pack. Finally, a bacterial suspension was inoculated to sterile Thioglycollate Broth to target 1.0x10⁸ CFU/mL and mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual glass slide carriers (1" x 1") per product batch, each in a Petri dish matted with two pieces of filter, were inoculated with 0.02 mL (20.0 µL) of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C and at 66% relative humidity. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction

so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 10 seconds at 20.5°C temperature and 8.7% relative humidity. Following the exposure period, the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Brain Heart Infusion Broth + 0.14% Lecithin + 1.0% Tween 80. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto BAP agar plates and were incubated at 35-37°C in a CampyPak™ Plus pack for 2 days. Following incubation, the subcultures were visually enumerated. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No amendments were made. Deviations reported in this study were reviewed and found to be acceptable.

20. MRID 493634-20, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)," Test Organism: Methicillin Resistant *Staphylococcus aureus*-MRSA (ATCC 33592), for product CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40 and 13wz41. Study conducted at ATS Labs by Joshua Luedtke, M.S. Study completion date – March 12, 2014. Project Number A16291.

This study was conducted against Methicillin Resistant *Staphylococcus aureus*-MRSA (ATCC 33592). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40 and 13wz41. These were tested using ATS Laboratory Protocol No. CX18010914.NFS.8 (copy provided). The product was received as ready to use saturated towelettes. From a stock slant, an initial 10 mL tube of Synthetic Broth growth medium was inoculated, mixed, and incubated, followed by performing a minimum of three daily subculture transfers, each incubated for 24±2 hours. The final subculture was incubated for 48-54 hours and was mixed thoroughly prior to use. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual glass slide carriers (1" x 1") per product batch, each in a Petri dish matted with two pieces of filter, were inoculated with 0.02 mL (20.0 µL) of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide in each Petri dish. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry with lids slightly ajar for 30 minutes at 35-37°C and at 41% relative humidity. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 10 seconds at room temperature (21.4°C) and 9.7% relative humidity. Following the exposure period, the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated on Tryptic Soy Agar plates with 5% Sheep Blood (BAP). All subcultures were incubated for 48±4

hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No amendments were made. Deviations reported in this study were reviewed and found to be acceptable.

Note: Antimicrobial susceptibility testing was performed by ATS Labs for Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592). The Kirby Bauer susceptibility assay was performed utilizing a representative test organism from the day of testing. After incubation at 35-37°C for ≥ 24 hours, the zone (diameter) of inhibition showing no visible growth was measured to verify the antibiotic resistance of the test organism.

21. MRID 493634- 21, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)," Test Organism: *Salmonella enterica* (ATCC 10708), for product CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40 and 13wz41. Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – March 12, 2014. Project Number A16284.

This study was conducted against *Salmonella enterica* (ATCC 10708). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batch 13wz40 and Batch 13wz41. These were tested using ATS Laboratory Protocol No. CX18010914.NFS.6 (copy provided). The product was received as ready to use saturated towelettes. From a stock slant, an initial 10 mL tube of Nutrient Broth growth medium was inoculated, mixed, and incubated, followed by performing a minimum of three daily subculture transfers, each incubated for 24 \pm 2 hours. The final subculture was incubated for 48-54 hours and was mixed thoroughly prior to use. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual glass slide carriers (1" x 1") per product batch, each in a Petri dish matted with two pieces of filter, were inoculated with 0.02 mL (20.0 μ L) of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide in each Petri dish. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry with lids intact for 35 minutes at 35-37°C and at 41% relative humidity. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 10 seconds at room temperature (22.0°C) and 9.1% relative humidity. Following the exposure period, the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated on Tryptic Soy Agar plates with 5% Sheep Blood (BAP). All subcultures were incubated for 48 \pm 4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No amendments were made. Deviations reported in this study were reviewed and found to be acceptable.

22. MRID 493634-22, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)," Test Organism: *Escherichia coli* O157:H7 (ATCC 35150), for product CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40 and 13wz41. Study conducted at ATS Labs by Kristen Niehaus, B.A. Study completion date – March 12, 2014. Project Number A16282.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40 and 13wz41. These were tested using ATS Laboratory Protocol No. CX18010914.NFS.4 (copy provided). The product was received as ready to use saturated towelettes. From a stock slant, an initial 10 mL tube of Synthetic Broth growth medium was inoculated, mixed, and incubated, followed by performing a minimum of three daily subculture transfers, each incubated for 24±2 hours. A final subculture was incubated for 48-54 hours. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. This was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual glass slide carriers (1" x 1") per product batch, each in a Petri dish, were inoculated with 0.02 mL (20.0 µL) of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide in each Petri dish. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry with lids intact for 32 minutes at 35-37°C and at 40% relative humidity. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 10 seconds at room temperature (21.6°C) and 23.2% relative humidity. Following the exposure period, the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Lethen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated on Tryptic Soy Agar plates with 5% Sheep Blood (BAP). All subcultures were incubated for 48±4 hours at 35-37°C, then were placed at 2-8°C for two days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: No amendments were made. Deviations reported in this study were reviewed and found to be acceptable.

23. MRID 493634-23, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)," Test Organism: *Streptococcus pyogenes* (ATCC 19615), for product CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40 and 13wz41. Study conducted at ATS Labs by Gracia Schroeder, B.S. Study completion date – March 12, 2014. Project Number A16302.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Testing was conducted using test substance CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024, Batches 13wz40 and 13wz41. These were tested using ATS Laboratory Protocol No. CX18010914.NFS.7 (copy provided). The product was received as ready to use saturated towelettes. A stock plate of the test organism was inoculated to multiple Tryptic Soy Agar plates with 5% Sheep Blood agar (BAP) and incubated for two days at 35-37°C in CO₂. Following incubation, a bacterial suspension was inoculated to Fluid Thioglycollate Medium to target 1.0x10⁸ CFU/mL and mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual glass slide carriers (1" x 1") per product batch, each in a Petri dish, were inoculated with 0.02 mL (20.0 µL) of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 41% relative humidity. Each towelette was prepared for testing by folding up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. One towelette was used to wipe the contaminated portion of 10 carriers. Each inoculated carrier was wiped with the saturated towelette by passing over the carrier surface back and forth twice for a total of 4 passes, using staggered intervals and then allowed to expose for 10 seconds at 21.0°C temperature and 9.4% relative humidity. Following the exposure period, the individual carriers were transferred using sterile forceps at staggered intervals to 20 mL of Brain Heart Infusion Broth + 0.14% Lecithin + 1.0% Tween 80. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto BAP agar plates and were incubated at 35-37°C in CO₂ for 48±4 hours. Following incubation, the subcultures were visually enumerated. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Note: No amendments were made. Deviations reported in this study were reviewed and found to be acceptable.

V. RESULTS

Table 1. Summary of Efficacy Test Results – Additional Bacteria

CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014				
MRID Number	Organism	Batch No.	No. of Carriers Exhibiting Growth/ Total No. Tested	Carrier Population (Log ₁₀ CFU/ Carrier)
3 Minute 30 Second Exposure Time				
49364-01	<i>Pseudomonas aeruginosa</i> (ATCC 15442)	13wz36	0/10	6.14
49364-02	<i>Pseudomonas aeruginosa</i> (ATCC 15442)	13wz38	0/10	6.10
49364-03	<i>Salmonella enterica</i> (ATCC 10708)	13wz38	0/10	4.58
49364-04	<i>Salmonella enterica</i> (ATCC 10708)	13wz36	0/10	4.50
49364-07	<i>Bordetella pertussis</i> (ATCC 12743)	13wz36 13wz38	0/10 0/10	6.72
49364-08	<i>Enterobacter aerogenes</i> (ATCC 13048)	13wz36 13wz38	0/10 0/10	6.53
3 Minute 35 Second Exposure Time				
49364-05	<i>Staphylococcus aureus</i> (ATCC 6538)	13wz36	0/10	5.55
49364-06	<i>Staphylococcus aureus</i> (ATCC 6538)	13wz38	0/10	5.44
CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013				
3 Minute 45 Second Exposure Time				
49364-09	Methicillin Resistant <i>Staphylococcus aureus</i> - MRSA (ATCC 33592)	12DMK1 12DMK2	0/10 0/10	6.64

Table 2. Summary of Efficacy Test Results – Additional Viruses (3min. 30sec.)

CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014					
MRID Number	Organism	3 Minute 30 Second Exposure Time			Dried Virus Count
493634-10	Avian Influenza A (H3N2) Reassortant virus, ATCC VR-2072		Batch No. 13wz36	Batch No. 13wz38	
		10 ⁻¹ to 10 ⁻⁸ dilutions	Complete Inactivation	Complete Inactivation	10 ^{4.50} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{1.50}	≤10 ^{1.50}	
493634-12	Avian Influenza A (H7N9), wildtype A/Anhui/1/2013, CDC #2013759189	10 ⁻¹ to 10 ⁻⁷ dilutions	Complete Inactivation	Complete Inactivation	10 ^{5.00} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{0.50}	≤10 ^{0.50}	
493634-13	Canine Distemper Virus (ATCC VR-128)	10 ⁻¹ to 10 ⁻⁷ dilutions	Complete Inactivation	Complete Inactivation	10 ^{5.50} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{1.50}	≤10 ^{2.50}	
493634-15	Duck Hepatitis B virus (surrogate virus for Human Hepatitis B virus)	10 ⁻¹ to 10 ⁻⁴ dilutions	Complete Inactivation	Complete Inactivation	10 ^{4.75*} TCID ₅₀ /0.25 mL
		TCID ₅₀ /0.25 mL	≤10 ^{0.50}	≤10 ^{0.50}	
		TCID ₅₀ /0.25 mL	≤10 ^{0.50}	≤10 ^{0.50}	10 ^{4.50**} TCID ₅₀ /0.25 mL
493634-16	Newcastle Disease Virus (ATCC VR-108)	10 ⁻¹ to 10 ⁻⁷ dilutions	Complete Inactivation	Complete Inactivation	10 ^{4.75} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{1.50}	≤10 ^{1.50}	

*Replicate 1

** Replicate 2

Table 3. Summary of Efficacy Test Results – Additional Viruses (3min. 45sec)

CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013					
MRID Number	Organism	3 Minute and 45 Second Exposure Time			Dried Virus Count
493634-11	Avian Influenza A (H5N1) virus, VN H5N1-PR8/ CDC-RG; CDC # 2006719965		Batch No. 12DMK1	Batch No. 12DMK2	
		10 ⁻¹ to 10 ⁻⁷ dilutions	Complete Inactivation	Complete Inactivation	10 ^{5.00} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{0.50}	≤10 ^{0.50}	
493634-14	Duck Hepatitis B virus (surrogate virus for Human Hepatitis B virus)	10 ⁻¹ to 10 ⁻⁴ dilutions	Complete Inactivation	Complete Inactivation	10 ^{4.75*} TCID ₅₀ /0.25 mL
		TCID ₅₀ /0.25 mL	≤10 ^{1.50}	≤10 ^{1.50}	

*Results for replicates 1 and 2 were the same

Table 4. Summary of Efficacy Test Results – Additional Bacteria (sanitizer claim)

CDQ, EPA Reg. No. 5813-79, F2012.0024 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0024					
MRID Number	Organism	Batch No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			Log ₁₀ CFU/Carrier		
10 Second Exposure Time					
49364-17	<i>Klebsiella pneumoniae</i> (ATCC 4352)	13wz40	<1.30	6.39	>99.9
		13wz41	<1.30	6.39	>99.9
		13wz44	<1.30	6.39	>99.9
49364-18	<i>Staphylococcus aureus</i> (ATCC 6538)	13wz40	<1.30	6.58	>99.9
		13wz41	<1.30	6.58	>99.9
		13wz44	<1.30	6.58	>99.9
49364-19	<i>Campylobacter jejuni</i> (ATCC 29428)	13wz40	<1.30	7.17	>99.9
		13wz41	<1.30	7.17	>99.9
49364-20	Methicillin Resistant <i>Staphylococcus aureus</i> -MRSA (ATCC 33592)	13wz40	<1.30	6.84	>99.9
		13wz41	<1.30	6.84	>99.9
49364-21	<i>Salmonella enterica</i> (ATCC 10708)	13wz40	<1.30	6.42	>99.9
		13wz41	<1.30	6.42	>99.9
49364-22	<i>E. coli</i> O157:H7 (ATCC 35150)	13wz40	<1.30	6.65	>99.9
		13wz41	<1.30	6.65	>99.9
49364-23	<i>Streptococcus pyogenes</i> (ATCC 19615)	13wz40	<1.90	7.83	>99.9
		13wz41	<1.78	7.83	>99.9

VI. CONCLUSIONS

1.) The submitted efficacy data **support** the use of the ready-to-use towlette containing CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014 as a disinfectant against the following bacteria on hard, non-porous surfaces with a 5% organic soil load for;

3 minute and 30-second contact time at room temperature (20-21°C), see Results; Table 1.:

<i>Pseudomonas aeruginosa</i> (ATCC 15442)	MRIDs 493634-01 and -02
<i>Salmonella enterica</i> (ATCC 10708)	MRIDs 493634-03 and -04
<i>Bordetella pertussis</i> (ATCC 12743)	MRID 493634-07
<i>Enterobacter aerogenes</i> (ATCC 13048)	MRID 493634-08

3 minute and 35-second contact time at room temperature (20-21°C):

<i>Staphylococcus aureus</i> (ATCC 6538)	MRIDs 493634-05 and -06
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3 minute and 45-second contact time at room temperature (20-21°C):

Methicillin Resistant <i>Staphylococcus aureus</i> -MRSA (ATCC 33592)	MRID 493634-09
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Acceptable killing was observed in the subcultures of the required number of carriers tested. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Antibiotic resistance of the Methicillin Resistant *Staphylococcus aureus* test organism was confirmed.

2.) The submitted efficacy data **support** the use of the ready-to-use towlette containing CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014 as a disinfectant against the following viruses on hard, non-porous surfaces with a 5% organic soil load for a 3 minutes and 30-second contact time at room temperature (20-21°C), see Results; Table 2.:

Avian Influenza A (H3N2), Reassortant virus, ATCC VR-2072	MRID 493634-10
Avian Influenza A (H7N9), wildtype A/Anhui/1/2013, CDC #2013759189	MRID 493634-12
Canine Distemper Virus (ATCC VR-128)	MRID 493634-13
Duck Hepatitis B virus, surrogate for Human Hepatitis B virus	MRID 493634-15
Newcastle Disease virus (ATCC VR-108)	MRID 493634-16

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Recoverable virus titers of at least 10^4 were achieved. No cytotoxicity was observed except in the Canine Distemper and Newcastle Disease virus tests. The reduction in viral titer in the Canine Distemper virus test was $\geq 4.00 \log_{10}$ and $\geq 3.25 \log_{10}$ in the Newcastle disease virus test. Complete inactivation (no growth) was indicated in all dilutions tested.

3.) The submitted efficacy data **support** the use of the ready-to-use towlette containing CDQ, EPA Reg. No. 5813-79, F2012.0013 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0013 as a disinfectant against the following viruses on hard, non-porous surfaces with a 5% organic soil load for a 3 minutes and 45-second contact time at room temperature (20-21°C), see Results; Table 3.:

Avian Influenza A (H5N1) virus, VNH5N1-PR8/ CDC-RG; CDC # 2006719965	MRID 493634-11
Duck Hepatitis B virus, surrogate for Human Hepatitis B virus	MRID 493634-14

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Recoverable virus titers of at least 10^4 were achieved. No cytotoxicity was observed except in the Duck Hepatitis B virus test. The reduction in viral titer in the Duck Hepatitis B virus test was $\geq 3.18 \log_{10}$. Complete inactivation (no growth) was indicated in all dilutions tested.

4.) The submitted efficacy data **support** the use of the ready-to-use towlette containing CDQ, EPA Reg. No. 5813-79, F2012.0014 and PJW-622, EPA Reg. No. 67619-9, lotion of F2012.0014 as a sanitizer against the following bacteria on hard, non-porous surfaces with a 5% organic soil load for a 10-second contact time at room temperature (20-21°C), see Results; Table 4.:

<i>Klebsiella pneumoniae</i> (ATCC 4352)	MRID 493634-17
<i>Staphylococcus aureus</i> (ATCC 6538)	MRID 493634-18
<i>Campylobacter jejuni</i> (ATCC 29428)	MRID 493634-19
Methicillin Resistant <i>Staphylococcus aureus</i> -MRSA (ATCC 33592)	MRID 493634-20
<i>Salmonella enterica</i> (ATCC 10708)	MRID 493634-21
<i>E. coli</i> O157:H7 (ATCC 35150)	MRID 493634-22
<i>Streptococcus pyogenes</i> (ATCC 19615)	MRID 493634-23

Acceptable killing was observed in the subcultures of the required number of carriers tested. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Antibiotic resistance of the Methicillin Resistant *Staphylococcus aureus* test organism was confirmed.

VII. RECOMMENDATIONS

1.) The label claims that the ready-to-use CDQ wipe is an effective disinfectant against the following organisms on hard, non-porous surfaces for a 4-minute contact time:

Pseudomonas aeruginosa (ATCC 15442)
Salmonella enterica (ATCC 10708)
Bordetella pertussis (ATCC 12743)
Enterobacter aerogenes (ATCC 13048)
Staphylococcus aureus (ATCC 6538)
Methicillin Resistant *Staphylococcus aureus*-MRSA (ATCC 33592)
Avian Influenza A (H3N2), Reassortant virus, ATCC VR-2072
Avian Influenza A (H7N9), wildtype A/Anhui/1/2013, CDC #2013759189
Canine Distemper Virus (ATCC VR-128)
Duck Hepatitis B virus, surrogate for Human Hepatitis B virus
Newcastle Disease virus (ATCC VR-108)
Avian Influenza A (H5N1) virus, VNH5N1-PR8/ CDC-RG; CDC #2006719965

These claims are acceptable as they are supported by the submitted data.

2.) The label claims that the ready-to-use CDQ wipe is an effective non-food contact sanitizer against the following organisms on hard, non-porous surfaces for a 10-second contact time:

Klebsiella pneumoniae (ATCC 4352)
Staphylococcus aureus (ATCC 6538)
Campylobacter jejuni (ATCC 29428)
Methicillin Resistant *Staphylococcus aureus*-MRSA (ATCC 33592)
Salmonella enterica (ATCC 10708)
E. coli O157:H7 (ATCC 35150)
Streptococcus pyogenes (ATCC 19615)

These claims are acceptable as they are supported by the submitted data.

LABEL RECOMMENDATIONS

- Multiple instances of "...disinfects...anywhere* –or- everywhere**"
As stated, the use of *anywhere* and *everywhere* is misleading and included sites not supported such as porous and soft surfaces. Either remove the language or qualify them directly without reference notations (i.e., "...anywhere indicated on the label, etc.).
- Page 4, first and second block with Sanitization instructions, add *non-food contact surfaces* to ...*hard, non-porous*. Claims referencing only sanitization should include non-food contact when describing acceptable surfaces.
- Page 4, first block, remove reference to *surfaces that may come into contact with food*.
- Page 5 and 6, for the claim, "Can help reduce the risk of cross-contamination [on treated surfaces]," remove "[]," as *on treated surfaces* is not optional and must be included as part of the claim for cross-contamination.
- Page 5 and 6, for the claim, "Cleans –and/or- Disinfects –and/or- deodorizes [all] [in one easy step]," remove [in one easy step] or specify *when following disinfection use directions*.
- Page 5, for the claim, "[Cleans –and/or- disinfects all in one step]," remove *all in one step* or specify *when following disinfection use directions*.
- Page 5 and 6, for the claim that appears in multiple instances, "Kills 99.9[99]%...", remove [99].
- Page 6, fourth bullet, qualify *Cold & Flu Viruses* as in the third bullet.
- Page 6, column II, there are multiple instances where in one step appears along with claim for cleaning and disinfecting, remove reference to *one step cleaning* in all instances.
- Page 6, *Antibacterial Claims continued*: "Quick and easy cart disinfecting," is

general and only applies to a subset of label claims supported with 10 second efficacy data. The use of the word *Quick* in reference to a public health claim (including marketing variations), 10 seconds has been the generally accepted maximum contact time. Only the specific claims supported with acceptable data using a contact time of 10 seconds or less may be specifically associated with the word quick. Quick appears in a similar claim with disinfection further down the same column and must also be removed along with any other instances in the label (marketing language that lacks any public health claims are excluded).